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NUTRITION REVIEWS

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NUTRITION REVIEWS

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GHANA: A CHALLENGE TO NUTRITIONISTS

Last Fall I had the professional good fortune of spending several weeks in Ghana. Together with Professor B. S. Platt, of the London School of Tropical Medicine and Hygiene, I was sent there on a general advisory mission by FAO and WHO. The opportunities for service by nutritionists in that country are so obvious, and both land and people are representative in so many ways of awakening Africa as a whole, that the Editor of *Nutrition Reviews* felt that the readers would be interested in brief observations on some of the outstanding nutritional problems of Ghana.

Ghana came into existence as an independent nation on March 6, 1957. Its territory had been administered previously as the Gold Coast, a British Crown Colony and as British Togoland, a Mandate. It is situated between 5° N and 11° N latitude and has an area of 92,000 square miles (about the same as Great Britain). Its population is under 5 million (corresponding to a population density similar to that of the United States, excluding Alaska). Ghana has remained for the time being in the Commonwealth of Nations but has entered into a loose federation with Guinea, which is not a Commonwealth member. The country is governed by a Prime Minister and Cabinet and a bicameral parliament with a house directly representative of the electorate and a house of Chiefs with more limited powers. It is divided administratively into five regions. Mr. Kwame Nkrumah has been Prime Minister since the country became independent.

The soils of the country are predominantly the end products of progressive surface disintegration of eruptive rocks, in particular laterite, a porous, clay-like brown, red or yellow rock high in ferrous oxide and aluminum hydroxide. Such soils are highly leached and potentially very productive if sufficient standards of management (including sufficient fertilizer application) are used. At present, however, methods of soil management are primitive and fertilizers are not in general use. From a nutritional viewpoint, it is useful to view the country as divided into four regions or population groups: (1) the arid coastal area, (2) the forest zone, (3) the northern savannah and (4) the large cities.

The mean annual rainfall varies between 60 and 100 inches in the forest zone, 30 and 60 inches in the southern savannah and frequently even less in the northern savannah. The arid coastal area is inhabited by the Ewes (particularly east of the Volta river), the Gas (in and around Accra, the capital) and the Fantis (particularly west of Accra). The staple foods of the Ewes are corn and cassava (manioc). Beans, oil (from oil palms) and fish (mostly from the Volta and its tributary) are frequent items of consumption. The Ewes thus are a relatively well-fed rural group, except where cassava represents too large a proportion of the diet.

The staple foods of the Gas people are also cassava and corn. Plantains, yams and bread (made from imported flour), egg plants, onions and palm nuts are frequent supplements. Beans and peas are relatively rare components of the diet. The supply of fish is mostly from the ocean and highly seasonal (about five months of the year). Meat is expensive, and eggs only occasionally eaten (and then not by the most "vulnerable" groups, *e.g.* children or pregnant or lactating women).

The diet of the Fantis is similar to that of the Gas, except that considerably more green leaves and somewhat more beans are consumed.

The agricultural population of the forest zone (which includes the territory of the Ashanti people and extends to the coast in the westernmost part of the country) is predominantly occupied in the growing of cocoa for cash and largely relies on imported food supplies. The main food crops are plantains and coco-yams, with cassava tending to replace some of these crops because it is so easy to cultivate. Corn, green leaves and palm oil are consumed in appreciable quantities. Rice consumption is limited to some areas. Fish and legumes are consumed in very small amounts.

The tribes of the northern region have very distinctive crops. Their staple foods are bullrush millet and sorghum. A variety of oil seeds and fermented locust beans and baobab seeds are frequent dietary items. Shea butter (from the seeds of *Butyrospermum parkii*) is an important item of export as well as of domestic consumption. Green leaves, eaten either fresh or powdered, are also important. Fish powder and guinea fowl are eaten not infrequently but in very small amounts. Cattle, goats and sheep are used for religious sacrifices and as a source of cash rather than as a source of meat. In most years, this area is subjected to a period of acute—and often dramatic—food shortage. Stores of foods are practically non-existent; if the rains fail, the only foods available may be wild leaves and other "bush foods". Sometimes even these fail.

The cities, Accra (the national capital), Kumasi (the capital of the Ashantis), and the western harbor of Sekundi Takoradi, have populations ranging from 50,000 to 200,000. The newly constructed eastern harbor and settlement of Tema is expected to develop soon a population of 80,000. The capital of the northern region, Tamale, is also growing rapidly. The Volta River Development project will require an industrial population. Before long over one tenth of the population will thus be urban. The foods consumed in the towns are still dominated by production in the surrounding territory, except that imported wheat flour plays an increasing role in the coastal cities and corn in Kumasi and Tamale.

Important infectious and parasitic diseases are malaria, tuberculosis, yaws, leprosy, trypanosomiasis (some forms of which are popularly known as "sleeping sickness"), onchocerciasis (the ocular localization of this filarial infestation causes "river blindness"), schistosomiasis (the fact that the agents, cercarial larvae, go through a stage where a snail is the obligatory host complicates irrigation developments) and guinea worms. Investigation and control of these diseases have been vigorously pursued by a remarkable organization of mobile medical field units.

The nature and frequency of prevalent nutritional diseases are not surprising in view of the dietary pattern described above. Malnutrition is widespread throughout the country. An indirect measure of malnutrition is the very high ratio of mortality of small children to infant mortality. In Ghana, more children die between the age of one to five than during the first year, an exceptionally unfavorable situation. While the sporadic famines in the North have attracted more attention, chronic protein deficiency throughout the country is the outstanding type of malnutrition. Throughout the country it results in anemia, which is so widespread that it is characterized as such in hospitals only if hemoglobin levels are below 50 per cent of normal. Hypochromic "iron deficiency anemia" appears to be far less due to parasitosis (including malaria) than is usually taught. It responds well to protein supplements (which may be devoid of iron, such as dry skim milk).

Incidence and prevalence of tropical ulcers is another indication of the extent of protein deficiency. As an example, at the time Dr. Platt and I visited Wa, a town in the northwest corner of the country, one third of the boys of the middle school were hospitalized for tropical ulcers. While malnutrition may not be the initial "cause" of

tropical ulcers it has been shown conclusively that these do not heal until patients are in strongly positive nitrogen balance.

The form and manner in which protein malnutrition expresses itself in young children in the various regions of Ghana is quite characteristically related to the availability of calories. Along the coast, average daily caloric intake approaches 2900 calories, which is probably nearly adequate, considering the size as well as the age and occupation distribution of the population. In the forest zone, surveys seem to agree on an average of 2800 calories per day per head which is again probably almost adequate. By contrast, caloric intakes are low in the North and yearly fall to starvation levels. Recorded regional averages of 1600 calories per day in July, fall to 1400 calories in December and to 1100 in February. In groups of sufficient sizes for averages to be meaningful, the highest monthly averages were 2065 and the lowest 675 calories per day. A number of deaths "due to starvation" have been recorded every few years.

In the southern regions (coast and forest region) where calories are abundant, kwashiorkor is the prevalent form of protein malnutrition in small children. In the northern region where calories as well as protein are likely to be short, marasmus dominates the clinical picture in infants and young children. Kwashiorkor is particularly full blown in the cassava-consuming areas of the forest region. This is not surprising in view of the fact that the extraordinarily low protein content of that root results in an average total protein intake of only 5 per cent of the calories consumed. Small children are usually denied even the small amounts of eggs and fish consumed by the men so that after weaning their protein intake is probably even lower.

Other features of malnutrition are vitamin A and perhaps riboflavin deficiencies in patchy areas and simple goiter in a region of the North where the iodine content of the waters and soil is presumed to be low and little or no sea salt is consumed. Though some of the European physicians in rural hospitals say that they have seen scurvy and beriberi, it was the impression of Dr. Platt and myself that these claims were due to inaccurate diagnoses. Bleeding gums, neuritis, and edema are common but did not seem to us to be due to ascorbic acid or thiamine deficiencies.

A highly encouraging feature of the situation is that the Ghana government and its high civil servants are extremely conscious of the importance of the nutritional situation. As an example of this understanding of the country's need, the prime minister said to me at the outset of a private conversation that protein deficiency was one of the most important problems Ghana had to face. Nor is the country without financial resources (Ghana produces one third of the world cocoa and an important fraction of the world manganese, and is developing a large hydroelectric aluminum-producing complex along the Volta). Technical personnel is available (the country has retained many of the ablest British technicians and administrators, attracted a number of foreign technicians and requested and obtained considerable technical assistance from the specialized United Nations agencies and bilateral assistance programmes). Ghana has an active university and a college of technology. These factors compensate in part for the paucity of trained nutrition workers and, with the proper impetus, should make for fast development in the administrative, agricultural, industrial and educational aspects of food production, processing and consumption.

It would be inappropriate for me to discuss in any detail the report embodying the recommendations of Dr. Platt and myself to FAO, WHO and the Ghana government as this document has not yet been made official. Suffice it to say that some of the points covered (which coincide with many of the plans already made by the government of Ghana) are the organization of an

overall food planning body, food technology, education programmes directed at young physicians, teachers, students and school children, industrial, school and preschool feeding, hospital dietetics, goiter prevention and a number of special projects.

The fact that programmes of such scope can be planned for Ghana is an indication of the maturity and enthusiasm of its leaders and administrators. It is likely that the rate at which progress will be made will depend on the number of trained nutrition and allied personnel, imported as well as local (and increasingly all-Ghanaan), that can be brought to work in the realization of such plans. Ghana thus offers one of the most interesting challenges to nutritionists in that conditions for success are exceptionally favorable, and successful measures developed there can be applied to many other areas of Africa south of the Sahara.

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THE PROTEIN ALLOWANCE CONTROVERSY

The absence of an allowance for protein intake for infants under six months of age in the current Recommended Dietary Allowances (National Academy of Sciences-National Research Council, *Publication 589*, Washington (1958)) signifies recognition of a long-standing area of inadequate information. The various points of view concerning a Recommended Dietary Allowance for protein for this age group are presented in the pediatric literature by two recognized authorities in this field (L. E. Holt, Jr., *J. Pediat.* **54,** *496* (*1959*) and H. H. Gordon and A. F. Ganzon, *Ibid.*, **54,** *503* (*1959*)).

In his article entitled "The Protein Requirements of Infants" Holt reviews pediatric history from the first valid analysis of protein shortly after 1880, at which time the different concentrations of protein in breast and cow's milk were first recognized. Scientific infant feeding of the subsequent era involved the dilution of cow's milk with the addition of carbohydrate.

Subsequently, the experiments of Osborne and Mendel demonstrated that, in the rat, casein was not as good a protein as lactalbumin and that cow's milk protein was not as good as that of breast milk. During the first two decades of the present century the concept was developed that 15 per cent of the calories should be derived from protein when cow's milk was fed. Shortly after 1920 processed milk became generally available and the problems of curd indigestion were solved by heat or acid treatment of whole cow's milk.

Further, Holt points out that, during the past two decades information has been acquired which would suggest that casein and lactalbumin apparently have equal nutritional value for the human infant; that nitrogen in processed milk is as available as that from breast milk; and that the net protein utilization for standard cow's milk protein and standard breast milk protein was 81.5 and 94.5 per cent, respectively, a difference which he considers surprisingly small.

Although there is a mass of clinical evidence that feedings containing ten per cent of the calories as protein (breast milk or simulated breast milks) or 15 per cent of the calories as protein (cow's milk formulas) are both eminently satisfactory, Holt explores the possibility that subtle differences do exist and reviews the indirect, and far from conclusive, evidence available. He notes that there is increased nitrogen retention on the higher protein intakes and that a part of the increased nitrogen retention represents "compensatory hypertrophy of tissues needed to handle the added nitrogen load." He also notes evidence for the retention of nitrogen not in the form of or-

ganized cellular protein such as urea and the amino acid accumulations within cells which might possibly function as "reserves."

The very important questions concerning the relative effects of the protein intake on resistance to infections, poisons, and the stresses of adult life in general have been reviewed. In his opinion answers to the question as to whether protein intakes of 2 g. or of 3 g. per kilogram per day provide the greatest margin of safety are as yet unknown.

In an accompanying article entitled "On the Protein Allowances for Young Infants" Gordon and Ganzon review the primary aim of the recommended dietary allowances "to maintain good nutrition in healthy persons in the United States under current conditions of living and to cover nearly all variations of requirements for nutrients in the population at large. These are meant to afford a margin of sufficiency above minimal requirements and are therefore planned to provide a buffer against the added needs of various stresses and to make possible other potential improvements of growth and function—they may be more generous than would be practical for feeding large groups under conditions of limited food supply or economic stringency." This frame of reference for the Recommended Dietary Allowances is not as well known as the stated allowances in the accompanying table.

Gordon discusses the three types of data used in formulating protein allowances; estimates of food intake and progress, theoretical formulations, and experimental studies of nitrogen balance.

In reviewing established artificial feeding practices of infants, Gordon feels that the recommendations of experienced pediatricians should be seriously considered because of lack of factual data and the lack of conclusiveness of the small amount of data available which compare the progress of full-term infants fed low or high protein intakes when the protein was derived from cow's milk. The cow's milk feedings used by the great majority of infants in this country supply from 13 to 16 per cent of the calories as protein and, assuming an intake of 150 ml. per kg. per day, supply approximately 3.5 g. of protein per kg. per day, which is the basis of the previous Recommended Dietary Allowance (1953 revision).

Actual intakes probably exceed these amounts of protein because of supplementary solids after the second or third month. V. A. Beal (*J. Nutrition* **50,** *223, (1953)*) has shown median protein intakes approximating 5 g. per kg. during the first six months of life in a group of economically privileged infants. Gordon was unable to find data in the literature on the progress of infants receiving cow's milk at a protein intake averaging less than 2.5 g. per kg. per day.

In regard to theoretical formulations of the protein requirements of infants, Gordon and Ganzon feel that there are many objections to such approaches, and that determination of protein allowances still requires actual testing of the effect on growth and health of the protein supplied in different diets.

Following a very careful review of the evidence concerning differences between breast and cow's milk and different levels of protein intake as evaluated by the nitrogen balance technique, they point out that the available data are only semiquantitative at best, and fail to distinguish the effects of high and low protein feeding on infants.

Examples of functional measures of dietary differences such as response to infection and changes in the sizes of organs are also considered. Does the renal hypertrophy on high protein feeding represent increased or decreased powers of adaptation? With due consideration of the inappropriateness of the recommended allowance of approximately 3.5 g. of protein per kg. in areas where cow's milk is not easily available for artificially fed infants, and because of the failure of the available evidence to contra-

diet the formulations of eminent American pediatricians, Gordon and Ganzon would have preferred retention of the previous allowance. At the same time they strongly recommend breast feeding for full-term infants whenever it is possible.

In commenting on these two articles editorially, L. F. Hill (*J. Pediat.* **54,** *545 (1959)*) points out that both Gordon and Holt reach essentially the same conclusions after their analyses of pertinent data, and agree that there is insufficient evidence from the work done thus far on which to base a firm judgment either for or against high or low protein feedings. He also points out that the issue is important to pediatricians because it represents an unanswered challenge in the field of infant nutrition. He also remarks that infants exhibit a surprising degree of adjustability to wide variations in composition of feedings so long as their intake is not limited, and that the present controversy may make physicians more aware of the complexities of infant feeding and the need for a more critical evaluation of what is prescribed. Finally, Hill recognizes the importance of specifying protein allowances for countries other than our own where the milk supply is not as plentiful and where minimal allowances rather than optimal allowances need definition.

In summary, it would appear that some of the factors involved in this "controversy" are: 1. A lack of pertinent information; 2. Differences in interpretation of the terms "requirement" and "allowances"; 3. A well expressed need for parameters of measurement that are functional, such as resistance to infection or other types of stress.

MOTTLING IN MECCA

An early symptom of excess fluorine (mottling of the teeth) has been reported for groups residing in Morocco, Tunisia and South Africa. In 1948, in Cairo, Egypt, D. El Tannir noted mottled enamel in patients who had come from Mecca in Saudi Arabia. When, in 1954, he was summoned to Saudi Arabia, he was able to investigate this phenomenon more closely (El Tannir, *Am. J. Pub. Health* **49,** *45 (1959)*).

The water supply of Mecca comes from three wells. Zamzam well is the foremost and most ancient source and is located in the very center of the city. Another source is Zobeida well 30 meters deep, at Mount Kara, 25 kilometers from Mecca. This water passes through the Arafa Mountain via a series of stone tunnels. In 1950 the Royal Family conducted the water of a new spring, 55 kilometers from Mecca, to the Holy City, to satisfy the increasing demand for an adequate water supply. This was given the name of "Aziziah" after the late king Abdul Aziz Al-Saud.

El Tannir obtained the following data from 115 patients in King's Hospital at Zaher, 33 inpatients in Mecca Maternity Hospital, 46 outpatients in Ajiad Hospital and 340 students in primary and secondary schools: age, sex, nationality, place of birth, water source, place of childhood, social class, extent of mottling (brown, yellow, white and mottling-free), extent of caries.

Although samples of water from various pertinent sources were collected, equipment for analysis for fluorine was inadequate so that reference had to be made to an official printed report of water analyses carried out in 1936 (H. H. Rashid, Ministry of Public Health, Egypt (1936)). In parts per million the analysis for Zobeida indicated: fluorine, 2; iron, 0.06; manganese, nil. The analysis for Zamzam showed: fluorine, 1.5; iron, 0.15; manganese, nil. For Zaafaran spring, a further source, the analysis was: fluorine, ?; iron, 0.1; manganese, nil.

Zamzam is occasionally used for drinking water, while Zobeida has been the main source of drinking water for hundreds of

years until the provision of the water from the new Aziziah source in 1950.

The percentage of mottling among the inhabitants of Mecca amounted to 70.5 per cent of the total. No sex or social class differences were noted. Among the inhabitants who were born in Mecca and spent their childhood there, the percentage amounted to 90.8 per cent in adults and 48 per cent in children (deciduous teeth). The author compares these figures with an examination carried out in Salida, Colorado, where fluorides were present (2 ppm) in drinking water. Ninety per cent of the total population was afflicted. Brown stained teeth were found in 9.7 per cent of the total inhabitants of Mecca; this may be compared with 4.1 per cent in Salida.

Permanent teeth were more affected with mottling than the deciduous ones. This finding might be attributed to the fact that infants in Mecca are breast fed. The mother's milk, which naturally contains considerable calcium, is not, however, expected to contain the same high concentration of fluorine (this is a point which the author feels is worth investigation). In addition, infants drink little water. Mottling in deciduous teeth is mostly of the white patches type, suggesting a milder condition of fluorosis. Milder cases in deciduous teeth showed only slight loss of translucency of the teeth.

In few cases were all the teeth affected, but inwardly the incisors were very clearly mottled, upper control incisors more than laterals and the upper incisors more than the lower incisors. In cases where all the teeth were affected, incisors usually showed brown manifestations. This color was rare in molars. Discolorations were either deep brown, yellow or chalky white patches. Outer glaze was present in most cases. Some cases, however, showed loss of glaze. No gross malformations were observed. No abnormal attrition was observed.

The percentage of caries-free persons among the total inhabitants of Mecca was 62.8 per cent as compared with 55.7 per cent for inhabitants who were born and lived their childhood outside Mecca and who did not show fluorosis. The author considers the latter figure as standard for Saudi Arabia. Sixty-two per cent of the inhabitants of Mecca were free of peridontal disease compared to 42 per cent free of this disease in inhabitants who were born and who had spent their childhood outside the city and who showed no fluorosis.

The white discoloration has been described elsewhere as lines, but in the cases observed in Mecca most of the white discolorations were present in patches similar to the brown and yellow ones. Superficially it would seem that the white color is due to a milder condition of fluorosis than the yellow or brown. For example, there is less water consumed during the pleasant winter weather in Mecca. This, however, did not satisfactorily explain the presence of normal, brown and white patches alternating horizontally at the same period of calcification. The discoloration could, however, be attributed to an imbalance of some material other than fluorine although its presence might be provoked by fluorine. The author feels that the following supports such a hypothesis: (1) Sporadic cases of mottled teeth occur in districts where an excess of fluorine in drinking water is not known to occur. (2) According to analyses carried out by J. H. Bowes and M. M. Murray (*Brit. Dent. J.* **60,** *556 (1936)*) the mottled teeth of inhabitants of Maldon contain 0.032 per cent fluorine in the enamel and 0.073 per cent in the dentine. Yet, mottling usually attacks enamel and only occasionally extends to the dentine. These authors have suggested that staining may be related to the content of iron. T. Ockerse and B. Wasserstein (*J. Am. Dent. Assn.* **50,** *535 (1955)*) have suggested that the stain is caused by the presence of manganese compounds in the enamel. Following up this suggestion, El Tannir had a spectrographic analysis made of mottled enamel from Mecca and normal enamel from Cairo and found that

the manganese lines—2576.1A, 2593.7A and 2605.7A were quite apparent in the spectrogram of the mottled tooth. The author is of the opinion that fluorine acts to "fix" the manganese in the teeth. Presumably, light mottling in a specific region could be due to a relative deficiency of either fluorine or manganese and heavy mottling may in some instances be due primarily to an excess of manganese.

FURTHER STUDIES OF PANTOTHENIC ACID DEFICIENCY IN MAN

The widespread distribution of pantothenic acid in foodstuffs makes the development of spontaneous human nutritional deficiency very unlikely in the United States. For a number of years W. B. Bean and his co-workers have been attempting to induce pantothenic acid deficiency in man by feeding purified diets low in pantothenate together with metabolic antagonists to pantothenate. The results obtained with omega-methyl pantothenic acid have been reviewed previously (*Nutrition Reviews* **13,** *36 (1955)*; **14,** *37 (1956)*).

Within several weeks human volunteers developed fatigue, apathy and malaise. Emotional and mental changes were seen frequently and disturbances of gastrointestinal function were noted. A tendency toward postural hypotension and tachycardia was common, and several of the subjects developed manifestations of disturbed neuromuscular function. Infection was unusually prevalent in the group. An impaired ability to acetylate para-aminobenzoic acid and to excrete 17-ketosteroids seemed to be present. The eosinopenic response to corticotropin was subnormal and abnormalities in carbohydrate metabolism were evident.

Because of shortcomings in the original experimental design, the attempt to induce pantothenic acid deficiency in man under somewhat different experimental conditions has been reported by R. E. Hodges, M. A. Ohlson and W. B. Bean (*J. Clin. Invest.* **37,** *1642 (1958)*). Six healthy young men served as the subjects for the experiment. After the initial control periods the pantothenate deficient diet was administered by stomach tube for a period of eight weeks. The formula differed from that which had been used previously in several respects.

First, the vitamin supplements provided only 75 per cent of the recommended dietary allowances. This was done because of the possibility that excesses of other vitamins might mask the features of pantothenate deficiency. Second, native casein was used as a protein source; in previous experiments the casein had been digested with pancreatin. Corn oil again provided the dietary fat. Third, the salt content was markedly changed by substituting sodium chloride for the large amounts of sodium bicarbonate in the earlier diet.

After the period of deficiency the subjects were observed for an additional period of two to four weeks when pantothenate was restored to the purified diet.

The six subjects were divided into three pairs. A "deficient" pair received the formula without alteration. Omega-methyl pantothenic acid was added to the formula of the "antagonist" pair. The "control" pair had 20 mg. of pantothenic acid added daily. Because of evidences of disturbed potassium metabolism in the earlier experiments, 1 g. of potassium chloride was added daily to the formula of one member of each pair.

The general symptoms of malaise, fatigue and mental changes developed in the "antagonist" pair. Somewhat later the "deficient" pair began to note similar symptoms. No significant alterations in pulse rates or

blood pressure measurements occurred. In the "deficient" pair the urinary pantothenate fell to less than 1 mg. per day.

The excretion of 17-ketosteroids remained unchanged in contrast to the lowering previously reported. This point is of considerable interest in view of the marked adrenal dysfunction in experimental animals with pantothenate deficiency. It is possible that the large amounts of para-aminobenzoic acid used in the earlier experiments might have altered the pattern of steroid excretion.

As another index of adrenal function, the circulating eosinophils were measured before and after the intramuscular injection of corticotropin. The baseline eosinophilia which had developed with the older dietary formula did not occur with the new formula. Again the degree of eosinopenia following corticotropin in the "antagonist" pair was subnormal. The significance of this observation, however, would be questioned by many now that more refined tests of adrenal functional reserve are available.

A prominent feature of the earlier studies from the Iowa group was the development of achlorhydria. This finding could not be substantiated in the present report. A depression of gastric secretion, when it did occur, was only transient. Also, no impairment in the acetylation of para-aminobenzoic acid could be documented.

The altered salt content of the new diet eliminated the alkalosis which had been a prominent feature with the old diet. Clinical evidences of potassium deficiency were not recognized even in the subjects not receiving potassium supplementation. The unexplained lowering of cholesterol levels in the plasma, which had been noted with the old formula, was again found. However, in the light of the great amount of experimental evidence which has appeared, this may be attributed to the large amount of corn oil in the formula. Glucose tolerance was not significantly impaired by the new formula.

It is no doubt discouraging to the authors that many of the objective indices which had been thought suggestive of pantothenate deficiency could not be confirmed by repetition. It would appear that some of the changes were dependent on abnormalities of the diet independent of pantothenate deficiency. The variability may also be attributable to the small number of individuals studied and the variable metabolic response to pantothenic acid depletion or antagonism. The degree of pantothenate deficiency induced in the human subjects must have been relatively small because many of the biochemical reactions requiring pantothenic acid containing coenzymes continued unaltered. The possibility that gastro-intestinal bacteria contributed pantothenate to the body cannot be excluded.

Despite the inconsistencies which have been discussed, there can be no doubt that a syndrome has been produced in man by pantothenic acid restriction and metabolic antagonism. It is hoped that the characteristics of the human deficiency state will be clarified further by a continuation of these painstaking studies.

AMINO ACID SUPPLEMENTATION OF A CORN DIET

It is recognized that a relative lack of one or more of the essential amino acids in a food protein reduces its biological value (*Metabolism* **6,** *350 (1957)*). Many animal experiments have demonstrated that such deficiencies can be corrected either by combining foods which mutually provide the missing amino acids, or by adding pure amino acids to the deficient protein. Such animal experiments have also revealed that on low protein diets the addition of too much of a single amino acid can result in

imbalance and in a reduction in biological value (*Nutrition Reviews* **14,** *129, 225, 311 (1956)*).

The application of such knowledge to the formulation of human diets adequate in protein has been hindered by lack of complete knowledge as to the optimum pattern of amino acids. The most widely used approach has been that of assuming that egg protein is of high biological value and therefore perhaps approaches more nearly the optimum amino acid pattern.

The FAO (Food and Agricultural Organization of the United Nations) committee on protein requirements has tried to solve the problem by using all available data in establishing a theoretical reference protein whose amino acid pattern would serve as a reference pattern (*Protein Requirements*, FAO Nutritional Studies No. 16, Rome, Italy (1957)). Whether or not such a reference amino acid pattern or reference protein will predict with accuracy the optimum protein needs of man under practical conditions will, of course, be influenced by such factors as the state of health of the individual (digestive efficiency) and the availability of amino acids in native proteins.

Two recent reports from the Institute of Nutrition of Central America and Panama (INCAP) Guatemala, C. A., represent attempts to evaluate the FAO reference pattern by using it as a basis for the supplementation of food proteins in nutrition studies with children recovering from severe protein malnutrition (N. S. Scrimshaw, R. Bressani, M. Behar and F. Viteri, *J. Nutrition* **66,** *485 (1958)*; Bressani, Scrimshaw, Behar and Viteri, *Ibid* **66,** *501 (1958)*).

The FAO reference protein pattern suggests that the eight essential amino acids should be supplied in the following amounts in milligrams per gram of nitrogen: isoleucine, 270; leucine, 306; lysine, 270; methionine and cystine, 270; phenylalanine and tyrosine, 180; threonine, 180; tryptophan, 90; and valine, 360.

The basal diet used by these workers consisted of corn-masa (lime-treated whole corn), 85 g.; corn gluten, 5 g.; glutamic acid, 2 g.; glycine, 3 g.; and cornstarch, 5 g. A multiple vitamin and mineral capsule was supplied daily to each child. This diet was analyzed and was found to be deficient in six of the essential amino acids on the basis of the FAO reference pattern. From the greatest to the least deficient, they arrayed as follows: tryptophan, lysine, methionine, valine, isoleucine, and threonine. Amino acid supplements were used, at the expense of glycine, in adjusting the basal diet iso-nitrogenously so as to equal the amino acid pattern of the FAO reference protein.

Two children served as the subjects and a total of 22 nitrogen balances (three-day) were conducted to assess the value of amino acid supplementation when the diet was fed at a level to supply 100 calories and 3 g. of protein per kilogram of body weight.

Although the number of trials was limited, the results were remarkably consistent. When the first two of the amino acids predicted to be deficient by comparison with the FAO pattern (tryptophan and lysine) were added to the diet in amounts to approximate that suggested for the pattern, nitrogen retention was improved markedly. When these two amino acids were added in excess (approximately 20 per cent excess), there was no detectable imbalance effect.

When methionine, the third limiting amino acid, was added to the basal diet plus tryptophan and lysine, it had a consistently adverse effect on nitrogen retention. The level used initially, however, exceeded the amount suggested by the FAO pattern by about 61 mg. per gram of nitrogen. When this correction was made, the adverse effect on nitrogen retention was eliminated, and strongly positive nitrogen balance was

achieved by further supplementation with isoleucine.

The further additions of valine and threonine tended to decrease nitrogen retention. However, the amounts of these amino acids used were again slightly in excess of the amounts suggested by the FAO pattern.

In these experiments it is clear that the diet was planned to supply a relatively liberal protein intake. Since some animal studies have indicated that differences in amino acid content of diets may be less critical at high intake levels, these workers also investigated the value of amino acid supplementation in a basal corn diet which supplied only 1.5 g. or 2.0 g. of protein per kilogram of body weight.

Qualitatively the responses noted were similar to those found in the previous studies when the total protein intake was higher. Step-wise supplementation with tryptophan, lysine and isoleucine, to match the FAO pattern, resulted in step-wise increases in nitrogen retention. Further supplementation with methionine again resulted in some decrease in nitrogen retention.

These interesting results suggest that the FAO pattern of amino acid requirements appears to represent a reasonable point of reference for evaluating the amino acid adequacy of a diet composed largely of corn. The possible exception appears to be that the suggested methionine level may be in excess of need. It is noteworthy that the simple diet used in these investigations appeared to be fully adequate, when supplemented with the deficient amino acids. The authors note that in one balance study the basal diet supplemented with three limiting amino acids (tryptophan, lysine, and isoleucine) gave better nitrogen retention than was observed when the same amount of protein was furnished by milk.

DIETARY EFFECTS ON HUMAN MILK FATTY ACIDS

Since the discovery that arterial lesions in atherosclerosis contain excess lipids, the diet has been suspected of accelerating this disease. Accordingly, research has attempted to classify dietary lipids either as helpful or harmful.

It was demonstrated by E. H. Ahrens *et al.* (*Lancet* **1,** *943* (*1957*)) that serum lipids reflect alterations in dietary fats. This evidence at least suggests that lipids in arterial walls may have come from lipids in blood, which in turn came from dietary lipids. Just how closely the detailed tissue pattern of fatty acid components matched the diet lipid counterpart could not be determined until recently. Now, silicic acid chromatography in conjunction with gas-liquid chromatography makes it possible to classify and quantitate the fatty acid spectrum in normal and pathologic tissue samples.

W. Insull, Jr., J. Hirsch, T. James and E. H. Ahrens, Jr. (*J. Clin. Invest.* **38,** *443* (*1959*)) studied the effects of rigidly controlled variations in diet on the nature of human milk fat. This metabolic endproduct has the advantage of being readily and frequently available. By deliberate alteration of diet it was possible to get information regarding such factors as synthesis of fatty acids, transfer of diet fat and transfer of depot fat to human milk lipids. Although information as to mechanisms of fat metabolism was sought, the specialized nature of human milk lipids precluded the application of information to other organs generally.

The patient submitting to the study was admitted to the metabolic ward on the fifth post partum day and remained for seven weeks. The mother's diet consisted of an orally administered liquid formula given

daily in five equal portions and supplemented with vitamins and minerals. The milk output was determined by weighing the baby before and after each feeding. At the end of each different diet period the entire 24 hour milk output was collected mechanically and mixed. Of this amount, 90 per cent was fed to the infant by bottle and the remainder was used for chemical analyses. Additional samples of four hour production were collected every other day.

The lipids were extracted using ethanol and ether. The solvent was evaporated and the residue was re-extracted with petroleum ether. A small amount of free fatty acid and traces of cholesterol and phospholipid were present, but since 98 per cent was triglyceride the lipids were not fractionated. Methyl esters of the fatty acids were prepared for gas-liquid chromatography. As a complementary technique duplicate samples were isomerized with alkali and analyzed using ultraviolet spectrophotometry.

Although about 4 per cent of the total acids present in human milk were of the C19–22 variety, only the C6–18 acids were considered in the present study.

The serum lipid classes comprising triglycerides, free and ester cholesterol and phospholipids were separated using silicic acid chromatography. Methyl esters of the fatty acids of esterified cholesterol, triglycerides and phospholipids were prepared and analyzed by gas-liquid chromatography.

The milk lipids must either be synthesized *in situ*, or transported either from fat depots or dietary fat. By varying the fat and non-fat calories in the diet and identifying the milk fatty acid pattern it was hoped that more information on the source of milk lipids could be determined.

Until lactation was established the patient was fed a regular hospital diet. After this, liquid formula feeding was used. Cow's milk protein at a level of 15 per cent of total calories was fed in all periods. In period two lard was used to provide 40 per cent of total calories. In period three no fat was fed and 1000 calories above the maintenance requirement were fed in order to promote fat synthesis from carbohydrate. In period four, 1000 calories less than the maintenance requirement were fed in a fat-free diet in order to promote the mobilization of depot fat and to be able to identify the depot fat pattern in milk fatty acids. In period five, corn oil was used at 70 per cent of total calories. An excess of 1000 calories was given in order to depress synthesis and transport of endogenous fat. In periods six and seven the caloric intake was reduced to a maintenance level and the calories from corn oil were 70 and 40 per cent, respectively. The purpose of these periods was to determine how the ratio of fat to carbohydrate affected the transfer of dietary fat into milk fat.

In periods one and two, palmitic and oleic acids predominated when the fat calories were 40 per cent of the total calories adequate for body weight maintenance.

In period three the lauric and myristic acid content doubled at the expense of stearic, oleic and linoleic plus linolenic acid. The palmitic and palmitoleic acids remained unchanged. The increase in lauric and myristic acids reflected the effect of feeding excess carbohydrate during a no-fat diet regimen. In period four, in which carbohydrate was decreased during a no-fat regimen, the fatty acid pattern resembled that seen during ad libitum feeding or when the 40 per cent lard diet was fed. The pattern resembled the fatty acid composition of depot fat reported by D. L. Cramer and J. B. Brown (*J. Biol. Chem.* **151,** *427 (1943)*). The feeding of a fat-free diet deficient in calories appeared to cause milk fatty acids to be derived from depot fat.

In period five through seven the corn oil diet resulted in a five fold increase in linoleic plus linolenic acids. In other words the pattern of milk fatty acids resembled the fatty acid composition of corn oil. The milk fat content of lauric and myristic acids exceeded the diet content in all feeding periods.

Odd numbered acids were less than 1 per cent. When lauric and myristic acids increased in period three, C13 and C15 were unchanged.

By the use of ultraviolet spectroscopy it was possible to differentiate the unsaturated fatty acids into mono, di, tri and tetraenes. Thus during the corn oil feeding the mixture which was determined as linoleic plus linolenic using gas-liquid chromatography was clearly identified as linoleic acid using ultraviolet spectroscopy. In fact trienes comprised only 1.4 per cent of total fatty acids, while trienes through hexaenoic compounds ranged around 2.5 per cent of the total fatty acids. There was no increase in tetraenes when dienes increased during corn oil feeding.

Serum cholesterol and phospholipid concentrations were highest on the lard and fat-free diets and lowest when corn oil was fed. Triglycerides increased when excess calories were fed with no fat in the diet. The milk lipids reflected the diet change within two days so that four day periods were adequate to demonstrate the effects. Serum lipids, on the other hand, required as long as two weeks to reach equilibrium with diet in long term experiments reported by E. H. Ahrens *et al.* (*Lancet* **I,** *943* (*1957*)).

Analyses of the fatty acid components of phospholipids, cholesterol esters and triglycerides of serum lipids revealed a difference in responsiveness to diet shifts. The triglyceride fatty acid pattern resembled the diet spectrum of acids most closely. Cholesterol esters contained more linoleic acid following the corn oil feeding. Phospholipids were not as susceptible to diet shifts. In this group 55 to 70 per cent of fatty acids were unsaturated with twice as much palmitic as stearic acid.

During the high-calorie, no-fat feeding in which lauric and myristic acids increased markedly in milk lipids, there was no parallel increase in these two acids in any of the ester groups of serum. Study of the fatty acid percentage composition of triglycerides of serum appears to indicate that this ester group might serve to transfer its fatty acids into milk.

The experiments demonstrate the ready transfer of diet lipids to milk fat. No clue as to the mechanism of production of increased amounts of lauric and myristic acids was afforded although several alternatives were considered. Oleic, stearic and palmitic acid equal 75 per cent of the fat of animals grown on a fat-poor diet. On the other hand, the absence of large amounts of lauric and myristic acids in serum ester fractions indicated that there was no evidence of transfer of these acids by serum. The authors suggested that the increased amounts of lauric and myristic acids would serve to reduce the melting point of milk glycerides. The presence of these fatty acids indicates that fat metabolism differs in the mammary gland compared with adipose tissue.

Summary: A study of the composition of fatty acids of milk during the feeding of lard, corn oil or no-fat diets was made. The human milk fatty acids reflected the composition of the fat that was fed. When no fat was fed, mammary synthesis of lauric and myristic acids predominated.

SELENIUM IN TEETH

Selenium first became of interest in nutrition because of toxic manifestations in grazing livestock occurring after the ingestion of certain plants with high concentrations of this element (S. F. Trelease and O. A. Beath, *Selenium. Its Geological Occurrence and Its Biological Effects in Relation to Botany, Chemistry, Agriculture, Nutrition and Medicine.* Published by the Authors, New York (1949)). While selenium concentrations in cereal crops usually do not exceed 100 ppm in seleniferous areas, "indicator" plants,

whose presence is routinely indicative of seleniferous soils, may concentrate selenium until levels of several thousand ppm are attained.

In contrast to these earlier studies on chronic selenium poisoning, more recent investigations have shown that trace amounts of the salts of selenium have clearcut abilities to protect rats against dietary liver necrosis and chicks against exudative diathesis (*Nutrition Reviews* **15,** *319 (1957)*; **16,** *149, 174, 319 (1958)*). These findings suggest that trace amounts of selenium are required for the normal nourishment of these species under the conditions of these trials.

In numerous ways, there is an obvious parallel between selenium and fluorides in the annals of nutritional history. Fluorides first became of interest in human welfare from a toxic standpoint with the recognition by F. S. McKay in collaboration with G. V. Black that excess amounts of a waterborne substance, later identified as fluoride, caused mottled enamel (*Dental Cosmos* **58,** *477, 627, 781, 894 (1916)*). Since that time a strong body of data has been collected which indicates the necessity of an optimal level of ingestion of fluorides during tooth development for the formation of highly caries-resistant teeth (*Dental Caries and Fluorine*, 1946, F. R. Moulton, Editor; *Fluoridation as a Public Health Measure*, 1954, J. H. Shaw, Editor, American Association for the Advancement of Science, Washington, D. C.).

Part of this caries-resistance, at least, is attributable to the incorporation of fluoride into the crystalline hydroxyapatite lattice in calcifying teeth at levels which are proportional to the amounts ingested (F. J. McClure and R. C. Likens, *J. Dent. Res.* **30,** *172 (1951)*).

Interest in selenium has also included studies on the teeth. In an earlier review, attention was drawn to epidemiologic studies conducted in Oregon where children born and reared in regions east of the Cascade mountain range, where the altitude is high and where there is a higher prevalence of bright days, had significantly lower caries incidence than children living in areas west of the Cascades where the altitude is low and the weather less bright (*Nutrition Reviews* **14,** *169 (1956)*). Additionally, a positive correlation was observed between the dental caries incidence and the level of urinary selenium excretion.

In more recent studies the urinary excretion of 79 boys in these areas of western Oregon was studied more extensively (D. M. Hadjimarkos and C. W. Bonhorst, *J. Pediatrics* **52,** *274 (1958)*). Twenty-four-hour samples of urine were collected. Thirty-three children lived in one county and 46 in the other. The average selenium concentration was 0.074 ± 0.007 ppm for the first group and 0.076 ± 0.005 ppm for the second group. The total amounts of selenium secreted were not given, although this would be helpful information. The individual concentrations varied from nearly zero to 0.199 ppm.

In the past, concentrations of over 0.050 ppm have been considered to be suggestive of toxic levels of ingestion. Yet in this study far more than half of the boys in each group had levels of excretion in excess of this value.

Selenium feeding trials with rats have not provided any definite answer as to whether the above correlation between urinary selenium excretion and caries-susceptibility may have a causative relationship. In the first study, rats were fed sodium selenite at daily doses varying from 0.2 mg to 1.0 mg per kilogram of body weight (M. G. Wheatcroft, J. A. English and C. A. Schlack, *J. Dent. Res.* **30,** *523 (1951)*). Definite systemic evidence of toxicity was observed without any statistically significant influence upon the dental caries incidence. However, a trend toward a higher incidence among the rats on the higher levels of selenium was reported.

In a second study no evidence of an increase in dental caries incidence was observed when sodium selenite was fed to a single group of rats at progressively in-

creasing levels from 10 mg to 30 mg per kilogram of diet as the rats increased in age and weight. General systemic evidences of toxicity were observed, but no pathology of the salivary glands was produced (J. C. Muhler and W. G. Shafer, *J. Dent. Res.* **36,** *895 (1957)*).

These studies did not take into account any possible influences of reduced dietary intake on dental caries incidence in the selenium-fed rats during the overt manifestations of toxicity. This reduced caloric intake during selenium toxicity has been repeatedly commented upon by investigators in the field. The well-known correlation between food intake and caries-susceptibility may have been involved in such studies and may have resulted in a lower caries incidence among the experimental rats than would have occurred if normal levels of food ingestion had been continued throughout the experiment (J. H. Shaw, *J. Am. Diet. Assn.* **24,** *181 (1948)*; Shaw, *J. Nutrition* **41,** *13 (1950)*; D. Bixler and J. C. Muhler, *J. Dent. Res.* **37,** *407 (1958)*).

Possibly still more important, these rodent studies were conducted after tooth development was complete. In all probability, any influence of selenium ingestion on caries-susceptibility in human beings may be due to the incorporation of selenium in the inorganic crystalline lattice of the enamel and dentin during development. No categorical statement can be made with respect to a causative relationship between selenium and caries-susceptibility until tests are made of selenium administration during tooth development.

Further suggestions to support this point are made by data in a paper by Hadjimarkos and Bonhorst (*Oral Surg., Oral Med., Oral Path.* **12,** *113 (1959)*), who analyzed teeth of individuals in Portland, Oregon, and found that appreciable amounts of selenium were present in enamel and dentin. The selenium values for various groups of permanent teeth varied from 0.38 to 0.52 ppm for dentin, and from 0.43 to 1.60 ppm for enamel. No increase in selenium values with age was observed. A small number of deciduous teeth was analyzed and values of 2.60 ppm for dentin and 4.50 ppm for enamel were observed.

No selenium values are yet available for teeth from areas where high and low amounts of selenium have been ingested for prolonged periods. These values along with correlations within the levels of urinary selenium excretion would be particularly valuable.

Relatively little is known of the metabolism of selenium in the animal body. Selenium is transmitted across the placenta of rats and cats when incorporated in the diet in either organic or inorganic form (B. B. Westfall, E. F. Stohlman and M. I. Smith, *J. Pharmacol. Exp. Therap.* **64,** *55 (1938)*). Likewise selenium passes across the mammary gland in cows and rats (M. I. Smith and B. B. Westfall, *Pub. Health Reports* **52,** *1375 (1937)* and K. P. McConnell, *J. Biol. Chem.* **173,** *653 (1948)*). In studies on the comparative levels of selenium in placental tissue and fetal cord blood, D. M. Hadjimarkos, C. W. Bonhorst and J. J. Mattice (*J. Pediatrics* **54,** *296 (1959)*) observed that tissues from 13 placentas contained an average of 0.18 $\pm$ 0.007 ppm of selenium while the fetal cord blood of the same cases contained an average of 0.12 $\pm$ 0.008 ppm. Unfortunately no samples of maternal blood were analyzed to permit a comparison between maternal and fetal blood.

It is interesting to note that these samples were collected in an area where the dietary content of selenium was very low. Valuable results would be obtained by extending these studies into seleniferous areas where appreciably higher amounts of selenium are present in the foods grown locally. It would be desirable to study the levels of selenium in the blood of different age groups in these areas in order to determine any variation which would explain the higher levels of selenium in deciduous teeth as reported by Hadjimarkos and Bonhorst (*loc. cit.*).

THE METABOLISM OF ADRENOCORTICAL HORMONES IN PREGNANCY

It is widely recognized that many important alterations in metabolism occur in pregnancy which are determined by ovarian, pituitary, placental and adrenal cortical hormones. There is now unfolding a body of information indicating that the metabolism of hormones themselves often is decidedly different in the pregnant state. New information concerning the metabolism of adrenal steroids will be considered in this review.

Measurement of the urinary excretion of corticosteroids has usually provided important information concerning corticosteroid metabolism. An increase in the excretion of glycogenic corticoids in the latter half of pregnancy was reported by E. Venning (*Endocrinology* **39,** *203* (*1946*)). Subsequent measurements of free cortisol excretion in pregnancy is consistent with this finding. A number of other workers who measured the urinary excretion of corticosteroids with chemical methods, which included both the free and the conjugated metabolic products of cortisol, found that there was no significant increase in the excretion of total corticosteroids.

When methods became available for the measurement of unconjugated 17-hydroxycorticosteroids, mainly cortisol, in the blood, a significant rise to about twice the normal level was observed during the third trimester in pregnant women. An exaggerated rise in the plasma cortisol takes place after the administration of ACTH.

A partial explanation for the failure to find an increase in total corticosteroid excretion in the face of high plasma levels was provided by C. J. Migeon, J. Bertrand and B. E. Wall (*J. Clin. Invest.* **36,** *1350* (*1957*)). These investigators demonstrated that the rate of disappearance of cortisol-4-C^{14} from the blood was greatly delayed in pregnancy. This observation was subsequently confirmed by M. Cohen, M. Stiefel, W. J. Reddy and J. C. Laidlaw (*J. Clin. Endocrinol. & Metab.* **18,** *1076* (*1958*)). The additional observation has been made by N. P. Christy, E. Z. Wallace, W. E. L. Gordon, and J. W. Jailer (*J. Clin. Invest.* **38,** *299* (*1959*)) that tetrahydrocortisol as well as cortisol has a prolonged half life in the blood of pregnant women. The impaired metabolism of cortisol differs from the impairment which occurs in cirrhosis of the liver, in which the metabolism of the reduced hormone is apparently unaffected.

Although the reported decrease in the rate of metabolism of cortisol helps to explain the pattern of the urinary excretion of corticosteroids, it fails to account for the elevation of the plasma level of cortisol and the absence of signs of hyperadrenal-corticism which would be expected with elevations of the plasma cortisol level.

An alteration in the physical state of cortisol in the blood during pregnancy has been described by W. R. Slaunwhite and A. A. Sandberg (*J. Clin. Invest.* **38,** *384* (*1959*)). Under certain conditions, plasma from pregnant women was shown by dialysis equilibrium to bind significantly more cortisol than plasma from non-pregnant women. The authors suggest that there is in pregnancy an increase in the plasma protein which normally is responsible for the binding of nearly all the cortisol of plasma.

The metabolism of aldosterone in pregnancy has attracted investigative interest because of the possibility that increased secretion of this hormone might be responsible in part for the salt retention which occurs in toxemia. Although the excretion of aldosterone in pregnancy has been measured by a number of methods, the most definitive study appears to be that of K. M. Jones, *et al.* (*Acta Endocrinologica* **30,** *321* (*1959*)).

Tritiated aldosterone was injected into six pregnant women and a similar number of women who were not pregnant to permit an estimation of the secretion rate of aldoster-

one by measuring the specific activities of aldosterone and its metabolites in the urine. The mean total secretion rate for aldosterone in the normal woman so measured was 192 μg per 24 hours. Most of the pregnant women were found to be producing larger amounts of aldosterone; the mean adrenal secretory rate was estimated to be 582 μg per 24 hours. In the urine of normal women there was only about 0.44 μg of unconjugated aldosterone, a modest increase to 1.7 μg was present in the urine from pregnant women. In contrast, there was an eight- to nine-fold increase in the aldosterone which is excreted in the urine as a labile conjugate which is hydrolyzed at room temperature at pH 1. This relative increase in the labile conjugate does not occur in the hyperaldosteronism which follows sodium restriction and may indicate an increase in this pathway of aldosterone inactivation.

Measurement of aldosterone in the blood of pregnant women was not technically feasible, but the authors believe that the unconjugated urinary aldosterone is a better index of free aldosterone in the plasma. Caution was urged in attempting to correlate alterations in sodium and potassium excretion with the excretion of the pH 1 labile conjugates.

Jones and his co-workers do not deny that there is an increase in aldosterone level in the blood in late pregnancy and they are sympathetic to the notion expressed by J. D. Martin and I. H. Mills (*Clin. Science* **17,** *137 (1958)*) that this increase is normally compensatory for the naturetic effect of progesterone.

In summary: The pregnant state is characterized by important changes in corticosteroid metabolism. Cortisol disappears from the blood more slowly. The limiting step may be at the stage of tetrahydrocortisol disposition. Elevated levels of cortisol in pregnancy are probably attributable to an increase in the concentration of a cortisol binding protein. Aldosterone metabolism apparently is not impaired in pregnancy and the formation of a labile complex may be favored. Previous concepts of the role of the adrenal cortex in pregnancy must be carefully re-examined in view of this new knowledge.

AMINO ACIDURIA IN KWASHIORKOR

The presence of amino acids in the urine has been detected in a number of unrelated illnesses (*Nutrition Reviews* **16,** *39 (1958)*). In normal individuals a relatively small number of amino acids are present in the urine in low concentration. The pattern in normal children is different than that in adults (*Ibid* **16,** *5 (1958)*). In an earlier qualitative study (*Ibid.* **16,** *259 (1958)*) amino aciduria was found during the early treatment of patients with kwashiorkor.

E. Schendel, A. Antonis and J. D. L. Hansen (*Pediatrics* **23,** *662 (1959)*) have reported data on the urinary excretion of amino acids in nine patients with kwashiorkor during the acute phase of the illness. Free amino acids were partitioned by the column chromatographic method from 24 hour urine specimens. The results were expressed as milligrams of amino acid per kilogram of body weight per day. The authors found that similar results were obtained when amino aciduria was expressed in relationship to the creatinine excretion, the total nitrogen excretion, or the percentage of intake.

Amino aciduria measured in four infants with kwashiorkor before treatment was 33.85 mg. of amino acids per kg. per day, a value which is 2.4 times the normal mean value of 14.23 mg. per kg. per day. Following a short period of treatment with milk, the mean excretion had fallen to 23.66 mg. per kg. per day. These increases were highly significant when compared with normal control infants. Expressed as the percentage

of intake excreted as amino acids the values during early treatment were 3.07 per cent compared with 1.26 per cent of the intake in normal children. The increased amino aciduria was not the result of the increased output of any particular amino acid or group of amino acids but was due to slight increases in all of the 17 acids measured except 3-methyl-histidine, alanine, and, perhaps cystine.

As might be expected from the variation in the degree of protein depletion in these patients there was considerable variability in the results. The amino acid excretion was greatly increased in one patient whose milk intake was increased by forced feeding and was then dramatically reduced during the second week of treatment.

Similar studies were carried out on three formulas composed of mixtures of synthetic amino acids. The first synthetic formula contained the 18 amino acids of casein, glucose, a mixture of minerals and all of the known vitamins. About half of the amino acids were present in the DL isomers in quantities twice that of the estimated requirement. The formula was fed in amounts isocaloric with the skimmed milk fed previously. A second amino acid formula consisted of the eight amino acids essential for adults plus tyrosine, arginine and histidine and contained fewer DL isomers. In this formula the amounts were based on available information concerning minimal requirements for infants, and 16 per cent of the calories were derived from amino acids against 59.5 per cent in the previous formula. A third amino acid formula was similar to the second except that the amino acids were all present in their natural isomers and the difference in amino acid concentration was made up with glycine so that it closely resembled the second formula.

On the synthetic amino acid formulas containing the DL isomers, amino acid excretion was very high in both normal infants and patients having kwashiorkor. In one of the patients it was noted that the mean excretion of L acids was 3.9 per cent of intake while 21.8 per cent of the intake of DL acids appeared in the urine. By considering the L and DL acids separately, patients with kwashiorkor on the synthetic amino acid diets had greater excretions of L amino acids in all four cases, while the excretions of DL acids were not increased compared with normal infants and actually fell below normal in two cases when these data were expressed as per cent of intake.

In two infants with kwashiorkor fed the synthetic diet containing the natural amino acid isomers, there was very little increase in amino aciduria when expressed on a weight basis or as per cent of intake. When the urinary amino acids were expressed as per cent of total urinary nitrogen, however, there was a doubling of amino acid excretion.

The amino aciduria seen during the first few days of treatment of kwashiorkor, using skimmed milk as the sole source of dietary protein, was a transitory phenomenon as are the renal dysfunctions sometimes seen in such patients. The extent of the excretion (3.1 per cent of the intake of nitrogen) was not large enough to interfere quantitatively in protein retention. However, it was thought to be of possible importance as an indication of general metabolic dysfunction. The fact that 17 different amino acids were found in the urine in almost equally increased amounts would suggest that no specific enzyme defect that has so far been recognized is responsible. This amino aciduria did not retard dramatic recovery of these infants either on natural or synthetic diets.

Amino acidurias of various types have been now identified in a great many nutritional and metabolic diseases. Although information is accumulating concerning the renal clearances of amino acids generally and specifically, in the present state of our knowledge it is difficult to assess either the qualitative or quantitative importance of

the amino aciduria reported in protein malnutrition. The very fact of its existence, however, would suggest that children with protein malnutrition may handle normal or high protein intakes in a different way than children who are adapted to such intakes.

ABSORPTION TESTS WITH I^{131}-LABELED FAT AND FATTY ACID

The diagnosis of steatorrheas has always been difficult because of the lack of simple and reliable means of measuring the separate processes of fat digestion and absorption. The collection of pancreatic juice by stomach tube is inconvenient and inaccurate, as are various determinations on 24-hour stool specimens. Various indirect tests have been unsatisfactory precisely because they have not measured the desired functions.

The availability of radioisotope tracer techniques, however, has made labeling with I^{131} practical, and a number of studies have been made with labeled fats and some with labeled fatty acids. Fat absorption usually has been determined by measuring the I^{131} content of blood, feces and urine after suitable test meals. A variety of conditions has been investigated (*Nutrition Reviews* **15,** *171* (*1957*)), including carcinoma of the pancreas, intestinal lipodystrophy, sprue, ulcerative colitis as well as patients after various surgical enterostomies and gastric operations.

Studies have also been reviewed on normal infants of various ages and in cystic fibrosis of the pancreas with and without ingestion of pancreatin (*Nutrition Reviews* **17,** *10* (*1959*)). Several investigators have used various iodinated dietary fats, e.g., corn oil. Others have used pure compounds, e.g., triolein.

It is clear that failure to split triglyceride should cause a readily detectable failure of fat absorption, while fatty acid absorption should be relatively unimpaired. On the other hand, normal fat digestion, but no fatty acid absorption, should occur if intestinal absorption is defective. Thus a labeled triglyceride and its labeled fatty acid represent a desirable pair of test substances.

D. Berkowitz *et al.* (*Ann. Int. Med.* **50,** *247* (*1959*)) have studied the blood absorptive patterns of these two compounds in a variety of clinical conditions and have evolved a simple and apparently reliable diagnostic test. The authors give a test meal containing 25 microcuries of I^{131}-triolein or oleic acid in 1 ml. of peanut oil per kg. of body weight in the fasting state, after blocking thyroid uptake by giving Lugol's (iodine) solution the day before the test. Serial venous blood samples are then taken until maximum blood radioactivity is reached. Another sample is taken at 24 hours. Blood levels are expressed as a percentage of the ingested dose, based upon an assumed blood volume. Determinations are also made on 24-hour urine and stool specimens. The authors point out that the radioiodine circulates in two forms, organic (attached to a lipoprotein molecule) and inorganic.

They have made over 500 studies and report on those conditions in which they find the labeled fat tolerance test valuable.

When plotted, the blood concentration curve in normal subjects reaches a peak at six hours of about 12 per cent absorbed, declining to about 2 per cent at 24 hours. The organic fraction (trichloracetic acid precipitable) is less than 50 per cent of the total. Berkowitz and co-workers find that blood concentration curves based on triolein alone or in combination with oleic acid are usually characteristic enough for identifying the patient's disturbance. Less often, further information from fecal analyses is needed. "Thus, a normal triolein curve rules out a major absorption defect, whereas a flat curve plus an elevated stool activity is malabsorption." The response to oleic acid is then used to distinguish faulty digestion

from a "primary mucosal defect." Thus a low flat triolein curve with a normal oleic acid curve indicates deficiency of pancreatic lipase. A flat curve for both test substances "suggests that the error is mucosal, as found in sprue." In the differential diagnosis of jaundice a normal triolein absorption curve serves to exclude biliary obstruction, which gives a flat triolein curve with a normal oleic acid test.

In evaluating the degree of fat malabsorption in various conditions the authors found the technique helpful. One interesting observation, aside from the expected findings in Whipple's disease, gastrocolic fistula and other known conditions with impaired absorption, was that patients with chronic passive congestion have a marked decrease in fat absorption and that this improves after cardiac compensation, perhaps because of the decrease in the edema of the intestinal wall.

The authors believe that studies with labeled lipids are of value in essential hypercholesterolemia, coronary atherosclerosis and perhaps obesity, as they have found marked differences from the normal in these conditions (delayed high peaks of total and lipid blood radioactivity and increased 24-hour values). Finally, they point out, the effects of other foodstuffs on fat digestion and absorption are easily measured (*e.g.* the depressed peak of blood levels when glucose is fed with the test fat meal).

Berkowitz and his colleagues have published simple, easily interpreted curves. The studies are practical in busy hospitals and avoid the need of the elaborate laboratory and collection routines usually found on metabolic research units. Their findings should have a fairly wide application and clinicians will benefit by improved diagnosis of various pancreatic deficiency and malabsorption syndromes.

It is ten years since S. J. Thannhauser and M. M. Stanley published "Serum Fat Curves Following Oral Administration of I^{131}-Labeled Neutral Fat to Normal Subjects and Those with Idiopathic Hyperlipemia" (*Tr. Assoc. Am. Physicians* **62,** *245 (1949)*). One wonders why further clinical application has taken so long.

In summary: The blood radioactivity curves after a test meal of I^{131}-labeled triolein and one of I^{131}-oleic acid distinguish readily between malabsorption due to impaired fat digestion (*e.g.* in pancreatic deficiency) and that due to a mucosal defect of absorption (*e.g.* in sprue).

DENTAL HEALTH IN THE CHILDREN OF NEWFOUNDLAND

The nutrition of the people of Newfoundland has been the subject of earlier surveys. A study conducted in August, 1944, established that the Newfoundlanders subsisted on a definitely lower level of intake than citizens of the United States or Canada (*Nutrition Reviews* **3,** *251 (1945)*). A high incidence of the clinical signs attributed to chronic deficiency of vitamin A, riboflavin and ascorbic acid was observed. No pellagra, no protein deficiency, and few signs of rickets or peripheral neuritis were encountered.

The same area was surveyed again five years later (*Ibid.* **7,** *331 (1949)*). A significant improvement in the clinical condition of the subjects was noted. This was attributed to increases in the available food supply, improved economic status, increased employment, decreased isolation, considerable activity on the part of various agencies directed toward improvement of nutrition, and to the fortification of margarine and the enrichment of flour.

During these surveys, comments were made on the high incidence of dental caries and other oral problems. In a recent study the incidence of dental caries and the quality of the diet were compared (T. Y. Lin and

M. D. Smith, *Canad. J. Pub. Health* **49,** *516* (1958)). The total number of teeth in the mouth, the decayed and missing teeth and the number of decayed tooth surfaces were noted as recorded for each of 400 children. These data were expressed as the "caries figure," which is the rate of caries to the total number of teeth in the mouth, i.e. the average number of decayed tooth surfaces per tooth.

The dietary information of the families as given by the mothers was noted for each factor studied as "daily," "frequently," "irregularly," "seldom" or "never" under the following headings: breast feeding, weaning formula, vitamin C supplement, vitamin D supplement, milk, citrus fruits, other fruits, vegetables, potatoes, meat, liver, fish, eggs, bread, cereal, cheese, pastry and candy. Arbitrary points were given to these various factors on the basis of the authors' conception of their relative importance: the poorest diets had nutrition scores by this system of 0 to 3, the fair diets 4 to 7, the average diets 8 to 11 and the good diets 12 to 15. The total range of scores possible under this system was from −6 for the worst combination of all factors to 24 for the best possible combination of all factors.

Altogether 282 children were given a dietary evaluation of this nature. The remainder were not so scored because of the unavailability of their mothers at the time of the examination. In the final statistical comparison, only the 128 children between the ages of 10 to 17 were included because the data for those younger contained the results for deciduous teeth as well and, hence, were uncertain due to the natural loss of these teeth.

In the statistical comparison of the average caries figures and average nutrition scores, there was a reduction in dental caries with improved nutrition. This reduction was significant at somewhat in excess of the 2 per cent level.

This is an interesting study, the validity and importance of which hinges on the accuracy of the diet history taken, as well as on the composite nutrition score developed by these authors.

METHIONINE AND HYPERTHYROIDISM

An interesting relation of methionine (and other sources of labile methyl radicals) to hyperthyroidism has been reported by R. E. Boldt, A. E. Harper and C. A. Elvehjem (see below.) The authors point out that hyperthyroidism entails increased metabolism of a variety of known nutrients, and that the hyperthyroid rat apparently requires at least one unidentified dietary factor found in a number of protein sources. The subject has been discussed in this journal (*Nutrition Reviews* **13,** *221* (*1955*)) but the ineffectiveness of methionine, there incidentally mentioned, may well have been under conditions of excessive metabolic need for labile methyl groups, as Boldt and co-workers indicate in their discussion. They point out that older nutritional work on labile methyl donors is often vitiated by deficiency of folacin and vitamin B_{12}, interfering with transfer or synthesis of these active radicals.

B. H. Ershoff (*Proc. Soc. Exp. Biol. Med.* **74,** *391* (*1950*)) found that the growth retardation of immature rats and mice fed large doses of thyroid powder was counteracted by a substance present in liver, kidney and "aureomycin mash". D. V. Tappan, Boldt and Elvehjem (*Ibid.* **83,** *135* (*1953*)) showed that the short survival of rats fed iodinated casein as a thyroactive material was prolonged by a number of natural products, including pork meat and alfalfa.

Boldt, Harper and Elvehjem (*Arch. Biochem. Biophys.* **74,** *437* (*1958*)) used groups of six weanling male rats fed a purified

ration containing salts, vitamins (including 0.125 mg. folacin, 0.01 mg. cyanocobalamin and 500 mg. choline chloride per 100 g. of ration), 5 per cent corn oil, 24 per cent protein (supplied by casein or cooked defatted pork), 0.4 or 0.5 per cent iodinated casein, and sucrose to 100 per cent. The iodinated casein served to make the animals "hyperthyroid". The experimental diets were given ad libitum and the survival times were noted up to 60 days. All groups gained weight for two weeks.

All rats of five groups on casein diets died before 60 days (average survival per group, 21.3 to 27.8 days). The pork eaters survived longer (17 of 30 rats survived to 60 days). A mixture of seven amino acids was added to the basal casein diet in two experiments, also with substantial increase in survival times (average 47.5 and 52.3 days; three rats survived 60 days).

When amino acids were tried individually as supplements (0.5 to 1.0 per cent) to the casein diet, DL methionine (0.6 per cent) showed the greatest protective effect (average survival 45.2 days; one rat survived 60 days). There was some protection when phenylalanine, serine or alanine was used. The authors point out, however, that growth was severely inhibited by methionine either alone or in the mixture; the casein contained 0.9 per cent methionine, giving a total of 1.5 per cent in the diet, a level known to retard normal growth. At a lower level, 0.3 per cent methionine supplementation increased survival without interfering with growth. Another methyl donor, betaine hydrochloride (0.3 per cent) was tried; survival was somewhat longer, but no animal survived 60 days in the experimental or control groups. Higher levels of this nontoxic substance were tried; at 1 per cent, mean survival was 46.4 days vs. 27.8 for the casein control animals; one rat lived to 60 days.

The authors point out that the methionine in casein and pork is similar in amount and availability. Although the survival of casein-fed animals approached that of the pork-fed group, it never equalled it. Thus, though labile methyl groups may well be a factor, the superiority of pork remains unexplained. On one hand, the diets contained plenty of choline as a source of methyl groups. On the other hand, casein (or roast beef) prevents the lactation failure, with low milk vitamin B_{12} levels, which develops in some rats fed pork, and it has been suggested that the latter may contain a factor interfering with utilization of this vitamin (*Nutrition Reviews* **10,** *219* (*1952*)).

The authors did not study the mode of action of methionine or other methyl donors and do not discuss this point. However, in a later paper (*Am. J. Physiol.* **195,** *175* (*1958*)), they test the hypothesis that methyl groups from the dietary choline might not be fully available because of impaired ability to oxidize choline to betaine. They measured the choline oxidase of rat liver homogenates and found a marked reduction in enzyme activity after 14 days of feeding iodinated casein. Their results "suggest that pork prolongs survival by partially preventing the decreased rate of choline oxidation associated with hyperthyroidism." Similarly, increased choline oxidase activity resulted from addition of diphosphopyridine nucleotide (DPN) to the homogenates from hyperthyroid rats and the authors speculate that the effect of dietary pork may be to prevent a fall in DPN concentration.

Other questions may be raised about the mode of action of methyl donors. Do these compounds enter in some way into the metabolism or utilization of thyroid substance or an analogue? Our previous knowledge of this field does not support this. Are the methyl groups serving to detoxify a thyroactive substance by methylation? Although this is a well known detoxifying mechanism available to the liver (*e.g.* for nicotinic acid) it is not known in the case of thyroxine or related compounds. Are the authors dealing with "antithyroid" substances? Probably not in the usual sense.

Antithyroid compounds of dietary origin are legion; they have usually been uncovered as causative agents in experimental goiter (*Nutrition Reviews* **16,** *19* (*1958*)) or in geographically localized goiter. For example, thyroid enlargement in a group of Tasmanian children has been found to result from drinking cow's milk containing goitrogen derived from turnips and other Brassicae in the cows' fodder (*Ibid.* **15,** *112* (*1957*)).

However, even "goitrogens" or "antithyroid" substances are not an homogeneous group; the whole subject can scarcely be resolved without pathologic and physiologic studies of the thyroid and these are missing from most nutritional papers. Thus, thyroid enlargement ("goitre") may be of quite different origins (*e.g.* colloid storage in an inactive iodine-deficient gland vs. hyperplasia in an overactive gland), and a given change can result from physiologic interference at quite different points in the hormonal and metabolic chain.

In summary: Methionine and other sources of labile methyl groups prolong the survival of rats fed thyroactive material (iodinated casein) and casein as a source of protein. The substitution of cooked pork for casein is still more effective. Liver choline oxidase activity is decreased in "hyperthyroid" rats, fed casein, as compared with those fed pork decreasing the availability of methyl groups from choline.

DIETARY PROTEIN AND SERUM CHOLESTEROL

The suspected relationship between serum cholesterol concentration and coronary artery disease in man has aroused interest in research on nutritional factors affecting serum cholesterol concentration; such studies represent an important segment of current nutrition investigations.

Among the many factors that have been shown to influence serum cholesterol concentration are the level and type of dietary fat (*Nutrition Reviews* **16,** *33, 68, 131* (*1958*); **15,** *1, 39* (*1957*); **14,** *67, 107, 193, 327* (*1956*)), the amount of cholesterol and cholic acid in the diet (*Ibid.* **16,** *42* (*1958*); **15,** *140, 227* (*1957*); **14,** *107* (*1956*)), the intake of plant sterols (*Ibid.* **14,** *39, 285* (*1956*)), and the dietary levels of a number of the vitamins (*Ibid.* **16,** *274* (*1958*); **15,** *119* (*1957*); **14,** *34, 47, 193, 245* (*1956*)). This by no means exhausts the list of dietary factors, to which may also be added endocrine factors, psychological factors and exercise.

It thus seems unlikely that any simple nutritional explanation for variations in the serum cholesterol concentration of man can be anticipated. Progress in obtaining some understanding of the relative importance of each of these factors can, however, be expected from the results of experiments on laboratory animals in which each variable can be studied independently. These can then serve as pilot experiments for more laborious and costly experiments on man.

Among the dietary factors that have received a minimum of attention from nutritionists may be included the level and the type of dietary protein. Attention has been drawn to the possibility of a relationship between protein intake, the incidence of coronary artery disease and serum cholesterol concentration by Yudkin (*Lancet* **2,** *155* (*1957*)) and by Yerushalmy and Hilleboe (*N.Y. J. Med.* **57,** *2343* (*1957*)). They have presented a figure showing that the correlation between mortality from degenerative heart disease in various countries and the grams of animal protein consumed per day is as good if not better as that previously shown between mortality and fat intake as claimed by A. Keys (*J. Mt. Sinai Hosp.* **20,** *118* (*1953*)).

The protein effect has also been re-

emphasized by R. E. Olson and his coworkers (Olson, J. R. Jablonski and E. Taylor, *Am. J. Clin. Nutrition* **6,** *111 (1958)*); Olson, J. W. Vester, D. Gursey, N. Davis and D. Longman, *Ibid* **6,** *310 (1958)*). This association aroused their interest in studying the influence of animal protein intake on serum cholesterol concentration in animals and man.

Another report by L. C. Fillios *et al.* (*Am. J. Physiol.* **194,** *275 (1958)*) is concerned with the relationship between the dietary level of protein and cardiovascular sudanophilia in the rat. This study was an extension of earlier work on the effect of diet on hypercholesterolemia (Fillios, S. B. Andrus, G. V. Mann and F. J. Stare, *J. Exp. Med.* **104,** *539 (1956)*), which has been supported by the work of others (R. J. Jones and S. Huffman, *Proc. Soc. Exp. Biol. Med.* **93,** *519 (1956)*; A. W. Moyer, D. Kritchevsky, J. B. Logen and H. R. Cox, *Ibid.* **92,** *736 (1956)*).

It is also interesting to note that when either cebus monkeys (Mann, Andrus, A. McNally and Stare, *J. Exp. Med.* **98,** *195 (1953)*) or rats (Fillios and Mann, *Metabolism* **3,** *16 (1954)*) are fed diets poor in methionine but high in choline they develop unusual hypercholesterolemia.

Olson *et al.* (*loc. cit.*) have been particularly concerned with the effect of methionine level and of choline intake upon the serum concentrations of various lipid fractions including cholesterol. Their first experiments were on young rats fed for two weeks on diets containing 18 per cent of protein either from a soybean protein, to give a diet low in methionine (0.16 per cent), or from casein, to give a diet higher in methionine (0.54 per cent). Cystine was added to the low-methionine diet to equalize the organic sulfur content of both diets. The choline and the fat levels in the two types of diets were varied. At the conclusion of each experiment blood was drawn and analyzed for various lipid and lipoprotein fractions.

Hypolipemia was observed in all rats fed on diets low in choline and methionine; in particular, the serum cholesterol values were lower than those for comparable rats fed similar diets that contained adequate quantities of choline. These effects were observed when the diets contained 8 per cent or 40 per cent of fat from lard, butter or corn oil. The addition of choline prevented the hypolipemia and resulted in elevation of the serum cholesterol concentration from 50 mg. per cent to 90 mg. per cent when the diet contained the soybean protein. The serum cholesterol values for rats on choline-deficient diets containing casein were higher (75 to 85 mg. per cent) and rose less than 10 mg. per cent when choline was added.

The authors suggest that the higher methionine content of the casein provided methyl groups for choline synthesis and that the lower serum cholesterol values observed in rats fed the diet containing soybean protein were primarily a result of a more severe choline deficiency.

An interesting sidelight of these studies is that the hypolipemia seen in choline deficiency was accompanied by fatty infiltration of the liver. The provision of choline reversed both of these effects, providing further indirect evidence that lipid transport is defective in choline-deficient rats (Rideout *et al. Biochem. J.* **58,** *306 (1954)*); Wilgram *et al. Circulation Res.* **5,** *111 (1957)*).

Olson *et al.* (*loc. cit.*) also studied the effect of the level of dietary protein on hospitalized human subjects. These patients received daily, during a control period, a diet providing 2400 calories and 1.0 to 1.2 g. of choline. This diet provided 100 g. of protein, mainly from animal sources, and 80 g. of fat daily; the remainder of the calories were from carbohydrate. The low protein diet provided 2400 calories also, but provided only 25 g. of protein, mainly from vegetable sources. It was considered to meet the minimal requirements of the subjects for all amino acids except methionine; also, it provided only about 0.2 g. of choline.

Each of the nine subjects who were fed the low-protein diet showed a fall in serum

cholesterol concentration during the experimental period of one week, from an average of 311 mg. per cent to 267 mg. per cent. This was accompanied by hypolipemia. There was no further decrease in one subject maintained for ten weeks on this diet. Another subject, also maintained for a longer time on the low-protein diet, showed a definite hypercholesteremic response when given either the high protein diet or a supplement of lipotropic factors including methionine, choline, inositol, liver extract and vitamin B_{12}.

In both the animals and the human subjects, a deficiency of lipotropic factors led to hypocholesterolemia which was reversed by a supplement of choline and methionine, thus implicating methionine, at least indirectly, as a factor in dietary proteins that can influence serum cholesterol concentration.

In the experiments of Fillios *et al.* (*loc. cit.*) mature female rats were fed on diets containing 23.6 per cent of fat, 1.5 per cent of cholesterol and 0.5 per cent of cholic acid, the remainder of the diet consisting of carbohydrate, either sucrose or starch, and casein. The casein level was varied from 5 to 60 per cent. The experimental period was 17 weeks, during which ten groups of eight rats each, four males and four females, were fed diets containing 5, 10, 20, 40 or 60 per cent of casein, with either sucrose or starch as the carbohydrate. Serum cholesterol concentrations were determined on each animal at three, six and 12 weeks and at the end of the experiment. Also the heart and aorta of each animal were stained with Sudan IV at the end of the experiment, and the degree of cardiovascular sudanophilia was taken as a measure of incipient atherosclerosis.

The serum cholesterol concentration of the male rats decreased from between 500 and 600 mg. per cent to about 275 mg. per cent when the protein level in the diet was increased from 5 to 20 per cent. Cardiovascular sudanophilia also decreased markedly, reaching a minimum when the diet contained 10 per cent of protein with starch and 20 per cent of protein with sucrose. Serum cholesterol values and cardiovascular sudanophilia in the female rats followed the same general pattern, except that all the values were higher. Increasing the protein level above 20 per cent led to a rise in cardiovascular sudanophilia in all cases. There was also a trend toward a rise in serum cholesterol concentration, but this was not as consistent, particularly in the female rats.

These experiments show quite clearly that the protein level of the diet influences both the serum cholesterol concentration and the deposition of fat in the heart and aorta of rats fed an atherogenic diet. It is of interest that with these atherogenic diets, which contained adequate choline, a low level of a protein primarily deficient in methionine led to elevated serum cholesterol concentration, whereas, in the experiments of Olson *et al.* (*loc. cit.*), a non-atherogenic diet containing adequate protein, but low in methionine and choline, led to hypocholesterolemia. Also, in the experiments of Olson and co-workers with human subjects, a low protein diet caused hypocholesterolemia. It should be noted, however, that the low protein diet in this case contained mainly proteins of plant origin, while the high protein diet contained proteins of animal origin. In view of the observations of Beveridge *et al.* (*Nutrition Reviews* **16,** *68 (1958)*) that plant lipids may carry some factor that depresses serum cholesterol concentration, it would seem important to determine whether plant proteins may not also carry some similar factor.

The observations of Fillios *et al.* (*loc. cit.*) that minimal cardiovascular sudanophilia occurs with a lower level of protein when the diet contains starch in place of sucrose may be related to the observation that a high level of sucrose in the diet depresses the food intake of rats fed inadequate quantities of protein (Harper and Spivey, *Am. J. Physiol.* **193,** *483 (1958)*). There is thus the possibility that the carbohydrate effect may in

reality be an indirect effect on protein intake.

In summary, these two series of experiments indicate that serum cholesterol concentration is influenced by the dietary level of protein, and that the protein effect may, in turn, be influenced by the concentration of lipotropic factors in the diet.

HIGH FAT DIETS AND BLOOD CHANGES

Approximately 20 years ago considerable work was done by V. Johnson and his associates at the University of Chicago on the destruction of red blood cells after the ingestion of fat. They found that during the absorption of fat (melted lard and olive oil), the lymph collected from the thoracic duct of dogs hemolyzed red cells when it was mixed with blood (Johnson and W. Freeman, (*Am. J. Physiol.* **124,** *466 (1938)*; *Nutrition Reviews* **1,** *408 (1943)*). In some cases almost half the red cells were hemolyzed. No such change occurred with lymph collected before, or seven hours after, the animals received the fat meal. The lymph collected under the latter circumstances was fairly clear, while that which hemolyzed the red cells was milky.

Freeman and Johnson (*Am. J. Physiol.* **130,** *723 (1940)*) suggested that the soap plus free fatty acids were responsible for the hemolytic action of the milky lymph. They found 3.3 to 6.3 mg. per ml. of free fatty acids in dog's lymph during rapid fat absorption. These levels are only slightly higher than those now being reported (for a review see D. S. Fredrickson and R. S. Gordon, Jr. *Physiol. Rev.* **38,** *585 (1958)*). The lymph from fasting dogs contained 1.0 to 2.0 mg. of free fatty acid per ml. This level, according to Freeman and Johnson (*loc. cit.*) was too low to produce hemolysis.

An extension of this work by A. Loewy, L. W. Freeman, A. Marchello and Johnson (*Am. J. Physiol.* **138,** *230 (1943)*); (*Nutrition Reviews* **1,** *408 (1943*; **4,** *83 (1946)*; **5,** *148 (1947)*) showed that when dogs were fed a high fat diet there was an increased excretion of bile pigment. This work was done using dogs whose common bile duct was anastomosed with the ureter. During the six to 16-day periods when the dogs received 5 to 10 ml. of corn oil per kg. of body weight, the excretion of bile pigment was from 20 to 100 per cent greater than on the alternate periods when the dogs were fed a low fat diet (canned dog food).

The above investigators concluded that the high fat diet hastened the destruction of red cells as evidenced by the increased bilirubin excretion. In spite of the apparent hemolysis, there was no change in the number of circulating red cells. They proposed that the red cell destruction by the chyle may play a role in the normal destruction of red cells in dogs.

Johnson, Freeman, and J. Longini (*J. Am. Med. Assn.* **124,** *1250 (1944)*) extended their observations to human subjects. They found that when blood from normal subjects was mixed with lipemic serum and distilled water there was a slight increase in the fragility of the red cells. However, when red cells from eight untreated pernicious anemia patients were treated in the same way, the red cells showed a much greater increase in fragility. On the basis of these observations, they suggested that the increased fragility of the red cells in pernicious anemia patients when exposed to lipemic serum may be one of the factors accentuating the anemia in that disease.

R. L. Swank has extended the work on the relation between high fat diets and changes in the blood (*Am. J. Physiol.* **164,** *798 (1951)*). He fed four healthy women a low fat diet (22 g. of fat per day) for two weeks. In the following two weeks the women received 150 g. of fat per day in addition to their usual diet. At the end of each week, a

fat tolerance was carried out after 15 hours of fasting. When 2 g. of fat (as cream) per kg. of body weight were given for the tolerance test, the chylomicrons tended to aggregate *in vitro* to a greater extent and for a longer period of time when the subjects received the high fat diet than when they were on the low fat diet. The aggregation of chylomicrons was studied in heparinized and citrated blood samples taken two, four, and six hours after the ingestion of the fat.

Swank's studies (*loc. cit.*) with six other adults showed that the tendency of the chylomicrons to aggregate was much greater following a test meal of butter fat (as cream), 1 g. per kg. of body weight, than when the same weight of olive oil (homogenized in skim milk) was given. Similar experiments with hydrogenated and unhydrogenated margarine oil, and a mixture of equal parts of linseed and cod liver oil, showed that the clustering of the chylomicrons was inversely related to the iodine number of the fat. The red cells behaved in a manner similar to the chylomicrons in that they showed an increased tendency to packing and distortion, and sometimes to clumping.

Similar studies with dogs (*loc. cit.*) suggested that even when the chylomicron count in the blood was decreasing, their tendency to aggregate was increasing (nine hours after the fat meal). Furthermore, Swank found with his limited number of subjects, that puppies and young adult dogs, as well as young adult human beings, showed a lesser tendency to aggregation of chylomicrons and red cells than older dogs and human subjects. The work with dogs also suggested that late in pregnancy, and for a short time thereafter, there was an increased tendency for the red cells and chylomicrons to aggregate after a fat meal.

R. L. Swank and C. F. Cullen (*Proc. Soc. Exp. Biol. Med.* **82,** *381 (1953)*) found that when hamsters were given a high fat meal, profound changes in the blood could be seen in the mucosa of the cheek pouches. They used 70 g. to 100 g. animals which had been maintained on mixed grains, greens and fox chow. To study the blood, different animals were anesthetized with urethane at various intervals after receiving 4 g. to 15 g. of butter fat (as cream) per kg. of body weight. One cheek pouch was inverted. A crescent-shaped incision was made through the epithelial layer which was then separated from the underlying tissue (according to technique of G. P. Fulton, G. Ruby, G. Jackson and B. R. Lutz, *Anal. Rec.* **96,** *554 (1946)*). The epithelial flap was turned over a glass block. The block and tissue were immersed in Ringer's solution maintained at 37.5 C.

Microscopic examination of the pouch flap from control hamsters which did not receive the test meal of fat never showed any stickiness or aggregation of red cells. There was no change in the red cells during the time when the lipemia was increasing (three to six hours after the fat meal). However, when the chylomicron count began to decrease (four to seven hours after the fat meal), the red cells began to adhere to one another. An occasional cell adhered to the capillary or venule wall. The speed of circulation decreased, with the flow of blood ceasing in many vessels. These changes were first evident, and remained most pronounced, in the venules. The red cells appeared to lose their contour in the aggregation process and frequently appeared to be covered by a "doughy" opaque material.

The blood began to return to normal seven to ten hours after the meal. This coincided with a clearing of the visible lipemia. A number of hamsters with the most pronounced blood changes were allowed to recover. Several days later when their pouches were examined, their blood supply appeared perfectly normal.

The more recent work of Swank (*Am. J. Physiol.* **196,** *473 (1959)*) indicates that when rabbits were given 3 g. to 5 g. of butter fat (as cream) per kg. of body weight, the animals became sluggish and their ears hyperemic. When the blood in the con-

junctiva of the animals was examined microscopically, 11 of 18 rabbits showed an abnormal aggregation of the red blood cells three hours after the cream meal had been given. The aggregation of the cells in the conjuntival blood vessels lasted some hours but was present in a few rabbits 24 hours after the fat meal.

The fat meal was associated with an increase in the circulation time as measured by the time required for appearance of Evans blue in the ear opposite to that in which it was injected. In fasting animals, the circulation time was eight seconds or less. It increased after the fat meal to an average of 12 seconds and remained elevated in the unanesthetized rabbits from the second to the sixth hour after the fat meal. From then on, it slowly returned to normal.

Significant changes in the shape and size of the red blood cells appeared in both rabbits and dogs given 3 g. to 8 g. of fat per kg. of body weight. Most of the abnormal cells became convex and developed rounded protuberances. Later the entire cell appeared to shrink and was covered by these protuberances. The normal biconcave red cell had a diameter of 7.5 to 8 μ, whereas the distorted cells had a diameter of 5 to 6 μ. A few cells became round and smooth and almost transparent. In this state, they did not aggregate. The above changes in shape were temporary. The normal circular shape and size was restored within hours after the fat meal.

There was a reduction of from 30 to 70 per cent in the platelet count in 24 experiments carried out in 16 rabbits following the high fat meal. In eight animals there were no significant changes.

There was a reduction in clotting time of 25 to 50 per cent in 16 experiments three to six hours after the rabbits received the dose of cream. The clots formed under these circumstances were gelatinous and friable. The fibrin strands which are normally present in the clot were absent in these. In four experiments there was no change in the clotting time.

There were no significant changes in the hematocrit or viscosity of blood from rabbits that had been fed the high fat meal. The buffy coat was much thinner in the rabbits that had been fed the fat. This change was apparent three to six hours after feeding. These changes in the buffy coat were reportedly seen in dogs and human beings after fat meals. This change was reported to be the earliest and most frequently encountered change in the blood after a fat meal.

The work of Swank and of Johnson and associates suggests that profound changes occur in the aggregation tendency of chylomicrons and red cells following a high fat meal. These changes can be seen both by *in vivo* observation and *in vitro* experiments. The clumping of the cells may progress to the point where the blood flow through small segments of tissue may be temporarily shut off. Most of the alterations in the blood disappear about nine hours after the meal. In animals such as rabbits, some abnormalities still may be seen 24 hours later.

These observations on the changes occurring in the blood of animals fed high fat diets are interesting and significant, coming as they do when so much attention is being focused on the effect of a high fat diet on the general metabolic reactions. It is hoped that an extension of this work will attempt to determine the long-term significance of the observed effects and whether these changes occur when a more complete diet high in fat is ingested.

NUCLEOTIDE INTERRELATIONSHIPS IN PROTEIN DEFICIENCY

There is little doubt that the nucleic acids are intimately involved in protein synthesis. Whether they serve as templates upon which to mold the myriad proteins of the body or

whether they are concerned with protein synthesis itself, possibly by activation of amino acids as the nucleoside phosphates, their role is of utmost importance (*Nutrition Reviews* **13,** *279 (1955)*; **15,** *55, 121 (1957)*). The consideration of a possible mechanism for the latter type of function reveals that it carries with it the implication that proteins, or at least amino acids, would also be concerned with synthesis of nucleic acids.

Some indication that this may be the case has been found in experiments with oocytes of protein-starved rats (*Nutrition Reviews* **16,** *304 (1958)*). However, the evidence has not been incontrovertible, and in certain microorganisms RNA synthesis can apparently continue after protein synthesis has been blocked (for a summary, see A. B. Pardee and L. S. Prestidge, *J. Bacteriol.* **71,** *677 (1956)*).

In order to clarify these relationships, S. Buch, J. D. Weil, M. Ledig and P. Mandel (*Bull. Soc. Chim. Biol.* **40,** *1487 (1958)*) studied the nucleic acids and nucleotides of the non-particulate portions of liver cells of normal and protein-depleted diets. The experimental animals were maintained on the protein-free diet until they had lost 30 to 40 per cent of their weight. The control animals, with 10 to 15 per cent dietary casein, grew normally. Animals were injected intraperitoneally with radioactive phosphate 24 hours before sacrifice and hepatic glycogen was reduced by a 12-hour fast. The trichloroacetic acid extracts of the non-particulate portions of liver cells were analyzed by chromatography on Dowex, charcoal and paper.

The total quantity of nucleotides was significantly lower in the protein-depleted animals, the ratio of concentration of nucleotides in starved animals to that in fed animals being about 0.6. However, the adenine nucleotides were almost normal, followed by the uridine and then the guanosine and inosine species. Within the groups, the monophosphates of the protein depleted animals were very low (ratio about 0.3), the adenine and uridine diphosphates were about normal, and the triphosphates were considerably higher than normal (ratios 4 to 5). Supplementations of the diets with glycine or aspartic acid had some effect, and a supplement of both these acids and glutamic acid brought the nucleotide values of the depleted animals close to normal. Supplementations with protein for three days brought the values to normal.

Parallel with these changes, the amounts of RNA and proteins decreased during the fast and increased somewhat with the amino acid supplementation. The specific activity of the RNA was high and decreased somewhat with supplementations. This latter observation was explained by the postulate of two fractions of RNA, one with a slow turnover rate, which disappears during a protein fast, the other with a high turnover rate and a consequent high activity.

The authors consider that their findings prove participation of proteins in RNA synthesis. In the protein-depleted animals, a large accumulation of nucleoside triphosphates, presumably the building stones for nucleic acids, appears as the nucleic acid and protein decrease. Supplementations with single, or even three, amino acids brings all values closer to normal. However, true normal values are obtained only with complete protein supplementation. The three amino acids selected are not members of the essential group, but aspartic and glutamic acids, being active in transamination, may have served as a source of most of the necessary amino acids. Other combinations of amino acids, including the essential members, should have been tried for more valid conclusions.

It is still uncertain whether the findings furnished proof of direct participation of amino acids in combination with nucleoside triphosphates as building blocks for nucleic acids, since concentrations of vital enzymes may also be lowered in protein deficiency. However, continuation of these studies may elucidate this complex and vital relationship further.

BERYLLIUM RICKETS AND VITAMIN D

The production of a type of rickets in the bones of growing rats through the addition of a beryllium salt to the diet was described by several investigators during the early thirties (H. D. Kay, *J. Biol. Chem.* **99,** *85 (1932)*; B. L. Guyatt, H. D. Kay and H. D. Branion, *J. Nutrition* **6,** *313 (1933)*; S. A. Jacobson, *Arch. Path.* **15,** *18 (1933)*).

Later the influence of a synthetic diet containing 3 per cent beryllium carbonate on the incisors of the young rat was reported by R. J. Gorin (*Oral Surg., Oral Med., Oral Path.* **4,** *177 (1951)*). The enamel showed no change but dentin was retarded in formation and calcification. A failure of resorption of the osteoid tissue occurred which led to a narrowing of the periodontal space on the convex side of the incisor and ultimately to a complete obliteration of this space.

More recently a detailed study was made on the effect of beryllium ingestion with different dietary levels of vitamin D on the rat incisor and its supporting structures (F. M. Wentz, I. Schour and J. P. Weinmann, *Ibid.* **11,** *1284 (1958)*). In addition, investigations were made on the calcium and phosphorus levels in the blood and the beryllium content of various organs. The rats were started on experiment at 24 days of age.

There were two major groups of rats with an experimental and a pair-fed control subgroup within each group. Twenty rats in the first group were fed a synthetic diet containing 300 units of vitamin D per 100 grams of diet and 6 per cent beryllium carbonate. The 20 littermates in the control subgroup were provided the same amount of the beryllium-free diet as had been consumed by the appropriate rat in the experimental subgroup. In the experimental subgroup of the second group, 20 rats were fed the same synthetic diet with beryllium carbonate and with enough added vitamin D to provide 2,670 units per 100 grams of diet. Nineteen littermate rats were maintained as pair-fed controls without the beryllium supplement. Pairs of rats were sacrificed in both groups progressively from 14 to 168 days on experiment.

Consistently the rats fed beryllium carbonate with the lower level of vitamin D, which was optimal for normal rats, had a severe rachitic syndrome. Enamel hypoplasia and aplasia were observed with severe retardation in the formation and calcification of dentin and a failure of newly formed bone to calcify and resistance to resorption. These changes were progressive up to the seventieth day, after which partial recovery was observed.

In the rats fed the same level of beryllium carbonate but the high, although sub-toxic, level of vitamin D, the rachitic process was much milder and was confined to rats under three months of age. It was noted that the rate of dentin apposition was slightly retarded, and calcification of lingual dentin was impaired with a slightly widened osteoid border in the alveolar bone.

The blood level of inorganic phosphorus averaged 10 mg. per cent at 59 days of age among the pair-fed control animals and gradually decreased to 5 mg. per cent at 192 days of age, which was considered to be within the normal range for rats of that age. The experimental rats in the first group had average inorganic phosphorus values in the blood of approximately 2 mg. per cent at 59 to 73 days of age. This level gradually increased thereafter to attain an average level of 5 mg. per cent by the end of the experiment, the same level as that of the comparable pair-fed controls. The inorganic phosphorus levels in the experimental rats in group two did not differ at any time from the values for the controls.

No beryllium was recovered from the incisor and its supporting tissues, nor from the kidney and liver. In the second group, beryllium determinations were made on the feces and levels of 1,360 micrograms were recovered per 150 milligrams of fecal material.

The authors particularly commented upon the parallel between the inorganic phosphorus values observed in the serum and the severity of the defects in calcification, and also upon the relationship of age to the severity of rickets. Their data support the opinion that beryllium poisoning is largely dependent upon a reduced absorption of phosphorus. However, in the presence of beryllium, the addition of greater than normal levels of vitamin D to the diet resulted in improved absorption of phosphorus.

The authors also commented upon the controversy in the literature concerning occurrence of hypoplasia in enamel during the rachitic process. The data in these new experiments indicate that the ability to produce hypoplastic enamel during rickets appears to revolve around the severity of the rachitic process with ample enamel hypoplasia in critically severe rickets.

This investigation appears to have been conducted in meticulous fashion and is liberally illustrated by photomicrographs of excellent quality. The conclusions make a substantial contribution to the field of rickets in general and beryllium toxicity in particular.

NOTES

Metabolism of Serum Albumin

The significance of serum albumin as a reserve of amino acids during periods of protein depletion has been investigated by N. S. Gimbel and C. Riegel (*Am. J. Clin. Nutrition* **6,** *142 (1958)*). They used a human volunteer who, in the first experiment, fasted for four days and then for two days was given intravenously 25 g. of amino acid nitrogen daily in the form of a fortified casein hydrolysate. This was again followed by a three day fast. In another experiment the procedure was similar except that the subject was given, intravenously, 12 g. of nitrogen from serum albumin daily for four days during the second period. Weight changes were followed, and the total nitrogen, urea nitrogen, potassium and sulfate excreted in the urine were measured.

Acetone was found in the urine after the first day and the respiratory quotient was about 0.7, indicating that the subject was using body fat as a source of energy. Following the administration of the casein hydrolysate, urea nitrogen excretion rose from 10 g. per day to 20 g. per day and sulfate excretion rose from about 550 mg. per day to 1200 mg. per day. This resulted in very little change in the ratio of urea nitrogen to sulfate in the urine.

After intravenous administration of serum albumin, urea nitrogen excretion fell from a value of about 9 g. per day during the preliminary period of fasting to about 4 g. per day. Urinary sulfate excretion increased by about 25 per cent. Thus the ratio of urea nitrogen to urinary sulfate fell from 19.5 to 9.3.

These results indicate that even under conditions of starvation intravenously administered serum albumin is not rapidly catabolized. In this respect the results are in agreement with previous studies on dogs (Howland and Hawkins, *J. Biol. Chem.* **123,** *99 (1938)*) and on surgical patients (Fletcher *et al. Surg. Gynec. and Obstet.* **90,** *151 (1950)*). The decrease in the urea nitrogen to urinary sulfate ratio during the period of albumin administration suggests that the sulfur from the albumin may be more rapidly catabolized than the amino acids generally. Serum albumin is high in sulfur-containing amino acids and such a result would be expected if the albumin that was utilized provided a relative excess

of cystine which was not needed for tissue synthesis. Nevertheless, the rise in sulfate excretion after the administration of albumin was much less than that observed after the administration of the hydrolysate, even though the albumin provided much more sulfur.

Calcium Ions and the Absorption of Vitamin B_{12}

Since it has been noted earlier that calcium ions are probably necessary for the absorption of vitamin B_{12} when given in physiological quantities, R. Grasbeck, I. Kantero and M. Srurala (*Lancet* **I**, *234 (1959)*) have suggested that the impaired vitamin B_{12} absorption in steatorrhea might be due to a deficiency of calcium ions. In steatorrhea the calcium is removed by formation of insoluble calcium soaps. By giving an oral dose of labeled vitamin B_{12} to six patients with steatorrhea (either idiopathic or due to celiac disease) 90 minutes before giving a 1 mg. flushing dose of non-labeled B_{12}, attempts were made to measure the absorption of the oral vitamin. The urine was collected for the next 24 hours and measured for radioactivity. Two tests were carried out; during one trial 7 g. of calcium lactate were given to adults and 2 g. were given to the infants. One half of the calcium lactate was given 30 minutes before the radiovitamin and the remainder was given simultaneously.

In all of the cases there was an increase of radioactivity in the urine when the patient received calcium lactate with the vitamin B_{12}, indicating that more of the test dose of the B_{12} was being absorbed when the calcium lactate was fed, though, in some cases, the differences were small. The authors suggest that there seems to be a deficiency of free calcium ions in the intestines of patients with steatorrhea, and this in turn might be responsible for the impaired absorption of vitamin B_{12}; though it should be noted that there are other mechanisms which may cause poor B_{12} absorption, such as achlorhydria, rapid passage of the intestinal contents or decrease in the area where absorption can occur.

In the case of six patients who had pernicious anemia, the administration of calcium along with the vitamin B_{12} caused no increase in the rate of absorption of the material. Thus, from these preliminary studies it appears that calcium ions played some role in the absorption of vitamin B_{12} from the intestinal tract in patients with steatorrhea, but not in those with pernicious anemia.

Recent Books

A Summary of the Symposium on the Chemical Basis of Development. Bentley Glass, McCollum-Pratt Institute. Pamphlet. The Johns Hopkins Press, Baltimore.

Nutrition and the Public Health, H. E. Magee, Ministry of Health. Pp. 143. Pitman Medical Publishing Co. Ltd., 39 Parker Street, London W.C.2., England; and 2 West 45th Street, New York.

Erratum

On page 121 of the April 1959 issue of *Nutrition Reviews* reference is made to a paper by R. L. Wixom and co-workers. *Nutrition Reviews* was in error in printing Dr. Wixom's name as Wixan.

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